

Improving Mucosal Barrier Function – A Novel Therapeutic Strategy for Crohn's Disease

a report by

Brian K Dieckgraefe

Assistant Professor of Medicine, Division of Gastroenterology, Washington University School of Medicine



Brian K Dieckgraefe is Assistant Professor of Medicine in the Division of Gastroenterology at Washington University School of Medicine in St Louis. He is also Director of the Functional Genomics Core Facility of the Digestive Diseases Research Core Center and Director of the Mentors in Medicine Program at Washington University School of Medicine. Dr Dieckgraefe serves on the Award Task Force Panel of the American Gastroenterology Association and is a member of the Crohn's and Colitis Foundation of America, among numerous other professional organisations. His clinical and research interests include gastrointestinal pharmacology and the intestinal epithelial response to injury and he has authored numerous publications in these areas. Dr Dieckgraefe completed a clinical and research fellowship in gastroenterology at Washington University School of Medicine and an internal medicine residency at Barnes Hospital in St Louis. He received his MD and PhD degrees from Washington University School of Medicine, having completed his undergraduate degree from the University of Kansas.

Crohn's disease (CD) is a chronic inflammatory disease that can cause ulcerations throughout the gastrointestinal (GI) tract, requiring surgery in approximately 70% of patients at some point during their lives. The aetiology of CD remains unknown, but the current hypothesis proposes that pathology results from an overactive, largely T-cell-mediated response directed against enteric bacteria. Local production of proinflammatory cytokines is believed to contribute to the chronic morbidity that is characteristic of this disorder and the increased presence of Th1 cytokines at sites of active disease supports this view. The prevailing therapeutic approach in clinical practice and ongoing clinical studies has therefore been to attenuate the excessive immune response using immunosuppressive agents, such as corticosteroids, azathioprine and 6-mercaptopurine, or antibodies targeting proinflammatory cytokines or cytokine receptors, such as anti-tumour necrosis factor (TNF)- α , anti-interleukin (IL)-12 and anti-IL-6 receptor. Such treatments are intended to diminish the downstream pathologic effector mechanisms that perpetuate CD, but do not address what may be the initiating cause of the disorder – compromised mucosal barrier function.

The intestinal mucosal barrier is composed of not only the mucosal epithelium, but also a highly integrated immune system. Recent evidence suggests that a defect in innate immunity may contribute to impaired intestinal barrier function. This article will discuss the role of innate immunity in mucosal defence and highlight evidence from recent clinical trials that demonstrates the therapeutic efficacy of granulocyte-macrophage colony-stimulating factor (GM-CSF) in the amelioration of CD.

Intestinal Mucosal Barrier

The first line of protection against enteric microbial infiltration is the physical barrier of the mucosal epithelium, composed largely of enterocytes, M-cells, goblet cells, Paneth cells and a luminal surface layer of mucus. Notably, the mucosal epithelium may play an important role in innate immunity, initiating the release of proinflammatory

cytokines in response to microbial exposure and providing regulation of the ensuing inflammatory response. Circulating components of the innate immune system, such as neutrophils, macrophages, dendritic cells, natural killer (NK) cells and complement proteins, are positioned beneath the basement membrane of this barrier to respond rapidly to translocating microbes and to stimulate the adaptive immune response (T-cells and B-cells). Induction of adaptive immune responses occurs primarily within localised structures of the gut-associated lymphoreticular tissue (GALT), whereas the effector response can occur throughout the lamina propria and immediately proximal to epithelial cells such as intra-epithelial lymphocytes.

The intestinal mucosa is normally in a steady state of regulated inflammation in response to continuous exposure to microbial and dietary antigens. The predominance of anti-inflammatory Th2 cytokines in the lamina propria of healthy individuals, produced in part by the interplay between regulatory T-cells and innate immune cells, contributes to immune tolerance towards this rich antigen supply. Dysregulation of tolerance and aberrant exposure to enteric microbes as a result of defective mucosal barrier function can alter the cytokine balance. The production of Th1 cytokines may contribute to chronic injury by perpetuating a vigorous adaptive immune response and inducing the local release of matrix metalloproteinases (MMPs). A defective mucosal barrier and dysregulated immune response ultimately alter mucosal permeability and subsequently increase antigenic exposure that eventually culminates in a vicious cycle.

Mucosal permeability is known to be increased in active CD due to the effects of inflammatory mediators, but there is conflicting evidence regarding the relative contribution of predisposing genetic and environmental factors to this defect. However, the increasingly clear association between the innate immune system and CD suggests that a failure to effectively clear intramucosal microbes may be central to the progression towards chronic transmural inflammation and tissue damage.

Evidence of a Link Between Innate Immunity and CD

The link between innate immunity and CD is supported by several genetic disorders with innate immune deficiencies that present a CD-like phenotype. Patients with a range of neutropenic disorders, including glycogen storage disease type 1b (GSD-1b), frequently develop GI disease indistinguishable from idiopathic CD. Also, impairments in critical functions of neutrophils, including migration, phagocytosis and superoxide production, have been demonstrated repeatedly in patients with active CD. Specific genes with important functions in innate immunity have also been associated with CD. Mutations in the NOD2/CARD15 gene were the first identified polymorphisms to confer susceptibility to CD. The NOD2/CARD15 gene is expressed by macrophages, dendritic cells and intestinal epithelial cells, and encodes an intracellular pattern recognition receptor that detects muramyl dipeptide (MDP), a bacterial peptidoglycan moiety. The most common CD-associated variant of NOD2/CARD15 was found to be non-responsive to MDP and functionally deficient in preventing intracellular bacterial survival. Moreover, mice that were deficient in this gene were found to be significantly impaired in controlling intestinal bacterial infection. Innate immune cells express toll-like receptors (TLRs), a family of pathogen-associated molecular pattern (PAMP) receptors that play a role in intestinal immunity. Polymorphisms in TLR4 and TLR9 have been associated with CD, and signalling through TLR2 was found to be modulated by NOD2/CARD15. Notably, peripheral blood monocytes (PBMC) from patients with the most common CD-associated NOD2/CARD15 variant demonstrated defective IL-10 release after stimulation with TLR2 ligands. The importance of IL-10 in suppression of intestinal disease is underscored by the high incidence of colitis in IL-10-deficient mice under normal but not germ-free conditions. Taken together, these findings indicate that innate immune deficiencies leading to impaired microbial clearance and disruption of normal Th2 cytokine production may result in a proinflammatory environment favouring chronic disease-promoting immune responses. It is becoming clear that the failure to effectively clear intramucosal bacteria due to impairments in mucosal barrier function is a central defect in CD.

Modulation of Innate Immunity and Mucosal Defence by Growth Factors

Several observations have suggested that therapeutic growth factor administration may attenuate the mucosal barrier function defect in CD. The endogenous growth factor GM-CSF exhibits

pleiotropic effects on myeloid cells, including the enhancement of microbicidal activity of phagocytic cells, such as macrophages and neutrophils, and the stimulation of expansion and differentiation of myeloid progenitor cells. GM-CSF also promotes the proliferation of colonic epithelial cells and is expressed at high levels in regions of the GI tract with the greatest luminal bacterial load (unpublished observations). The results of studies using transgenic mice with deficiencies in GM-CSF or the β_c -chain of the GM-CSF receptor have demonstrated the critical role this growth factor plays in mucosal innate immune defence of the lungs. Growth factor therapy has shown promise in amelioration of inflammatory bowel disease (IBD). Patients with the neutropenic GSD-1b disorder respond favourably to treatment with granulocyte (G) CSF. A small open-label study of G-CSF therapy for active CD also suggests some clinical activity. Successful therapeutic growth factor modulation of haematopoietic cell activity has also been observed in the prevention of graft-versus-host disease (GVHD) following allogeneic haematopoietic stem cell transplantation (ASCT). In one study, receipt of peripheral blood progenitor cell (PBPC) allografts mobilised with GM-CSF was associated with a reduced risk of acute GVHD compared with PBPC mobilised with GM-CSF plus G-CSF or G-CSF alone.

“Unlike GM-CSF, G-CSF has been suggested to act through a number of other immunomodulatory pathways, possibly functioning not as a stimulant of innate immunity alone but also to suppress certain aspects of immune response.”

Overall, these findings further support the potential clinical use of growth factor therapy in the treatment and prevention of disease.

Clinical Evaluation of Sargramostim Therapy for Active CD

Sargramostim (Leukine[®]), a recombinant version of GM-CSF, is used for myeloid cell reconstitution following chemotherapy in patients with acute myelogenous leukaemia (AML). Sargramostim was selected for evaluation in the treatment of active CD because of its potent effects on promoting the expansion and enhancing the functional activity of innate immune cells.

Pilot Study

The efficacy and safety of sargramostim therapy for CD was first evaluated in an open-label, dose-escalating pilot study of 15 patients with active moderate to severe disease (a baseline CD activity index (CDAI) score of >220 and <475). Patients were enrolled into one of three groups to receive a dose of 4, 6 or

8µg/kg/day of sargramostim by self-administered, subcutaneous injection. The primary efficacy measurement was the CDAI score of disease severity. Clinical response was defined as a >70-point decrease in baseline CDAI score and remission was defined as an absolute CDAI score of <150. A secondary measurement of treatment efficacy was a 32-item IBD questionnaire (IBDQ) that assessed quality of life. Fifteen patients were screened and enrolled in the study, all of whom completed the eight weeks of treatment. A rapid progressive decrease in the mean CDAI score was observed during the study. After eight weeks of sargramostim treatment, 12 patients (80%) achieved a clinical response and eight patients (53%) experienced remission. The mean CDAI score at eight weeks was 156 (standard deviation (SD): 97), a 190-point reduction in the mean baseline CDAI score ($p<0.0001$). All of the responding patients had a >100-point decrease in CDAI score after eight weeks of treatment and none of the non-responders had a significant increase in CDAI score. A substantial improvement to health-related quality of life (measured by an increase in IBDQ score) correlated with the significant reduction in disease severity following sargramostim treatment. After eight weeks of sargramostim treatment, the mean IBDQ score of all enrolled patients increased significantly to 164 ($p<0.0001$), and the mean IBDQ score of the clinical responders was 179 (SD: 22). In general, the daily sargramostim injections were well tolerated. The majority of patients reported medullary bone pain (67%) and minor injection-site reactions (80%); however, a single oral dose of acetaminophen before sargramostim injection provided relief for most patients and both adverse events markedly diminished over time.

Phase 2 Placebo-controlled Study

The favourable results of the previous pilot study were supported by findings of a recent multi-centre randomised placebo-controlled phase 2 study. A total of 124 patients with active moderate to severe CD (baseline CDAI score >220 and <475) were randomised (2:1) to receive 6µg/kg of sargramostim or placebo (81 and 43, respectively) by daily subcutaneous injection for eight weeks. Efficacy measurements included the evaluation of disease severity (CDAI score) and health-related quality of life (IBDQ score). Adverse events were recorded to evaluate the safety and tolerability of sargramostim injections. Sargramostim treatment significantly decreased the severity of CD relative to placebo. Upon study completion (day 57), significantly more patients in the sargramostim group achieved a clinical response (>100-point reduction in baseline CDAI score) compared with the placebo group (48% versus 26% respectively, $p=0.01$). The percentage of patients who achieved disease remission (defined as an

absolute CDAI score <150) was significantly higher in the sargramostim group than in the placebo group (40% versus 19%, $p=0.01$). Disease severity was re-evaluated in the majority of patients in both groups 30 days after study completion, at which time the sargramostim group maintained significantly higher rates of clinical response ($p=0.01$) and disease remission ($p=0.02$) compared with the placebo group. The reduction in disease severity correlated with an improvement in quality of life, as patients in the sargramostim group experienced a significant increase in IBDQ score above baseline compared with patients in the placebo group ($p=0.02$). Mild to moderate injection-site reactions and bone pain were more common in the sargramostim group. The incidence of injection-site reactions decreased after the second week of treatment, and bone pain was generally transient and manageable with acetaminophen. Sargramostim treatment resulted in a stable elevation in neutrophil and eosinophil counts during the study, the levels of which returned to baseline following cessation of treatment.

Conclusion

CD is likely to be a polygenic disorder with a common pathologic manifestation; however, the close association of innate immune deficiencies with chronic, CD-like intestinal pathology suggests that such immunodeficiencies may be central to this condition. Defective intestinal innate immunity may allow persistent exposure of cells of the lamina propria to enteric microbes and promote a pathologic, T-cell-mediated inflammatory response. In contrast to conventional immunosuppressive interventions that target this later stage of CD, sargramostim therapy may address the fundamental defect of this disorder by enhancing mucosal barrier function. The mechanism of action for the successful clinical response to sargramostim may relate to the potent effects of GM-CSF in promoting the expansion and microbicidal activity of neutrophils and macrophages, potentially enhancing microbial clearance and altering the local cytokine milieu. Considering the proliferative response of intestinal epithelial cells to GM-CSF, however, this growth factor could also play a larger role in maintaining intestinal homeostasis and repair. ■

Dr Dieckgraefe reports having received consulting and lecture fees from Berlex. A US patent entitled "Stimulating Neutrophil Function to Treat Inflammatory Bowel Disease (6500418)" was issued on 31 December 2002. Drs Korzenik and Dieckgraefe are the inventors. The patent is owned by Washington University School of Medicine and licensed by Schering AG, Germany.

A version of this article containing references and figures can be found in the Reference Section on the website supporting this business briefing (www.touchbriefings.com).